REMARKS

Reconsideration of this application is respectfully requested.

Claims 30, 31, 56-61, and 109-132 have been canceled (claims 109-132 were added by Amendment during Interference No. 102,822). New claims 133-141 are derived from canceled claims 30, 31, and 56-61 and are fully supported by the specification. Upon amendment, claims 133-141 are pending in this application. No new matter enters by amendment.

As requested by the Examiner, applicants submit herewith a copy of claims 30, 31, 56-61, 109-115, and 132, which were involved in Interference No. 102,822. Applicants have also enclosed a copy of the Judgment in Interference No. 102,822 (Paper No. 300), which indicates that applicants were judged not to be entitled to a patent containing claims 30, 31, 56-61, 109-115, and 132, corresponding to Counts 2-6. Applicants have also enclosed copies of Paper Nos. 271, 282, and 289 in Interference No. 102,822, which provide the language of Counts 2-6.

Claims 30, 31, and 56-61 were rejected under 35 U.S.C. § 102(e) as allegedly being anticipated by Chang et al. (U.S. Patent No. 6,001,977). The Examiner alleges that Chang discloses the claimed nucleic acids.

Applicants have canceled claims 30, 31, and 56-61. Applicants' new claims 133-141 recite that the DNA comprises a complete HIV-1 LTR. U.S. Patent No. 6,001,977 of Chang is a division of application No. 06/693,866 ("the '866 application"), filed January 23, 1985, which is a continuation-in-part of application No. 06/659,339 ("the '339 application"), filed October 10, 1984, which is a continuation-in-part of application No. 06/643,306, filed August 22, 1984. The '339 application (Exhibit 1), filed

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October 10, 1984, does not contain the sequence of a complete HIV-1 LTR. Rather, as indicated on page 9 and in Fig. 1 of the '339 application, an approximately 200 bp fragment of the HIV-1 LTR is ***missing*** from the clones. As a result, Chang could not disclose a complete HIV-1 LTR sequence until the '866 application was filed on January 23, 1985.[1] This is ***after*** applicants' foreign priority date of November 16, 1984, for Appln. GB 84 29099, in which applicants disclose a complete HIV-1 LTR sequence. The Office has acknowledged applicants' claim for priority of Appln. GB 84 29099 under 35 U.S.C. § 119. (*See* Paper No. 8.) A certified copy of Appln. GB 84 29099 was filed in this application on October 21, 1993. (*See* Copy of U.S.P.T.O.-stamped postcard receipt attached as Exhibit 2.) Consequently, Chang is not effective prior art with respect to applicants' claimed sequence. Accordingly, applicants respectfully request withdrawal of the rejection.

Applicants respectfully submit that this application is now in condition for allowance. In the event that the Examiner disagrees, he is invited to call the undersigned to discuss any outstanding issues remaining in this application in order to expedite prosecution.

Please grant any extensions of time required to enter this response and charge any additional required fees to our deposit account 06-0916.

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[1] None of the counts in Interference No. 102,822 was drawn to a DNA comprising a complete HIV-1 LTR. (*See* Paper Nos. 271, 282, and 289 in Interference No. 102,822.)

Respectfully submitted,

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Dated: March 7, 2003

By: _____

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CLONING AND EXPRESSION OF HTLV-III DNA

Description

Technical Fields

This invention is in the fields of biology and virology and in particular relates to human T cell leukemia virus - type III (HTLV-III).

Background Art

The term human T cell leukemia-lymphoma virus (HTLV) refers to a unique family of T cell tropic retroviruses. Such viruses play an important role in the pathogenesis of certain T cell neoplasms. There are presently three known types of HTLVs. One subgroup of the family, HTLV-type I (HTLV-I) is linked to the cause of adult T-cell leukemia-lymphoma (ATLL) that occurs in certain regions of Japan, the Caribbean and Africa. HTLV-type II (HTLV-II) has been isolated from a patient with a T-cell variant of hairy cell leukemia. M. Popovic et al., Detection, Isolation, and Continuous Production of Cytopathic Retroviruses (HTLV-III) from Patients with AIDS and Pre-AIDS, Science, 224:497-

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HTLV-type III (HTLV-III) has been isolated from many patients with acquired immune deficiency syndrome (AIDS). It refers to prototype virus isolated from AIDS patients. Groups reported to be at greatest risk for AIDS include homosexual or bisexual males; intravenous drug users and Haitian immigrants to the United States. ~~homosexual or~~ ~~bisexual males~~ having hetero~~sexual~~ contacts,

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hemophiliacs who receive blood products pooled from donors and recipients of multiple blood transfusions are also at risk. Clinical manifestations include severe, unexplained immune deficiency which generally involves a depletion of helper T lymphocytes. These may be accompanied by malignancies and infections. The mortality rate for those with AIDS is high. A less severe form of AIDS also exists, in which there may be lymphadenopathy and depressed helper T cell counts; there is not, however, the devasting illness characteristic of full-blown AIDS. There are many individuals, who are classified as having early AIDS (pre-AIDS), who exhibit these signs. It is not now possible to predict who among them will develop the more serious symptoms.

Much of the evidence implicates HTLV-III as the etiological agent of the infectious AIDS. First, there is consistent epidemiology; greater than 95% of the patients with AIDS have antibodies specific for HTLV-III. Second, there has been reproducible identification and isolation of virus in this disease; more than 100 variants of HTLV-III have been isolated from AIDS patients. Third, there has been transmission of the disease to normal healthy individuals who received blood transfusions from infected blood donors.

HTLV-III has been shown to share several properties with HTLV-I and HTLV-II but also to be morphologically, biologically and antigenically distinguishable. R.C. Gallo et al., Frequent Detection and Isolation of Cytopathic Retroviruses (HTLV-III) from Patients with AIDS and At Risk for AIDS. Science, 224:500-503. (1984). For example,

HTLV-III has been shown to be antigenically related to HTLV-I and HTLV-II by demonstrating cross-reactivity with antibodies to HTLV-I and HTLV-II core proteins, P24 and P19, and envelope antigens and by nucleic acid cross-hybridization studies with cloned HTLV-I and HTLV-II DNAs. However, unlike HTLV-I and HTLV-II, it lacked the ability to infect and transform T cells from normal umbilical cord blood and bone marrow in vitro, and has the cytopathic effect on infected cells only.

Like the RNA genome of other retroviruses, the RNA genome of HTLV-III contains three genes which encode viral proteins: 1) the gag gene, which encodes the internal structural (nucleocapsid or core) proteins; 2) the pol gene, which encodes the RNA-directed DNA polymerase (reverse transcriptase); and 3) the env gene, which encodes the envelope glycoproteins of the virion. In addition, the HTLV-III genome contains a region designated Px, located between the env gene and the 3' LTR, which appears to be involved in functional killing of the virus.

At this time, AIDS is still difficult to diagnose before the onset of clinical manifestations and impossible to treat or even prevent.

Summary of the Invention

This invention is based upon applicant's cloning of HTLV-III DNA in recombinant/vector host systems capable of expressing immunoreactive HTLV-III polypeptides. In one embodiment, an immunoreactive protein coded for by an env gene sequence of HTLV-III has been produced by these recombinant

DNA methods. This polypeptide is immunoreactive with sera of patients having acquired immunodeficiency syndrome or antibodies to HTLV-III. The polypeptide expressed has been isolated.

In another embodiment of the invention, immunoreactive polypeptides produced by the recombinant DNA methods are employed in the production of antibodies, including monoclonal antibodies, reactive with the polypeptides. Such antibodies form the basis for immunoassay and diagnostice techniques for detecting HTLV-III, particularly in body fluids such as blood, saliva, urine, etc.

In another embodiment of the invention, DNA probes are formed from DNA sequences coding for portions of the HTLV-III genome. Such DNA probes can also be employed in detecting the presence of HTLV-III in blood or other fluids.

Diagnostic kits including immunoreactive polypeptides, DNA probes, etc. can also be produced to include any of the products of this invention.

Brief Description of the Figures

Figure 1 is a representation of HTLV-III DNA. Figure 1a shows sites at which the genome is cut by the restriction enzyme SstI and Figure 1b shows the fragments of HTLV-III genome produced through the action of restriction enzymes Kpn, EcoRI and Hind III.

Figure 2 is a representation of HTLV-III DNA and the location of restriction enzyme sites in the genome.

Figure 3 shows nucleotide sequences for HTLV-III DNA which encompasses the env region.

Figure 4 is an immunoblot showing the position on an SDS polyacrylamide gel of HTLV-III env-Beta-galactosidase fusion proteins.

Best Mode of Carrying Out the Invention

The envelope glycoprotein is the major antigen recognized by the antiserum of AIDS patients. In this respect, HTLV resembles other retroviruses, for which the envelope glycoprotein is typically the most antigenic viral polypeptide. In addition, the neutralizing antibodies are generally directed toward the envelope glycoprotein of the retrovirus. Serum samples from 88 percent to 100 percent of those with AIDS have been shown to have antibodies reactive with antigens of HTLV-III; the major immune reactivity was directed against p41, the presumed envelope antigen of HTLV-III. Antibodies to core proteins have also been demonstrated in serum of AIDS patients, but are evidently not as effective an indicator of infection as is the presence of antibodies to envelope antigen.

The p41 antigen of HTLV-III has been difficult to characterize because the viral envelope is partially destroyed during the process of virus inactivation and purification. The present invention responds to the great need to characterize the antigenic component of the HTLV-III virus--and thus provide screening, diagnostic and preventive products and methods--in several ways.

First, the present invention relates to the isolation of genes of HTLV-III which encode

immunoreactive polypeptides; identification of the nucleotide sequence of these genes; introduction of DNA sequences specific to these viral DNA sequences into appropriate vectors to produce viral RNA and the formation of DNA probes. These probes are comprised of sequences specific to HTLV-III DNA and are useful, for example, for detecting the same HTLV-III DNA sequences in body fluids (e.g., blood).

Second, the present invention relates to HTLV-III polypeptides which are produced by translation of the recombinant DNA sequences encoding HTLV-III proteins. Polypeptides which are so produced and which are immunoreactive with serum from AIDS patients are referred to as recombinant DNA-produced immunoreactive HTLV-III polypeptides. They include, but are not limited to, antigenic HTLV-III core and envelope polypeptides which are produced by translation of the recombinant DNA sequences specific to the gag and the env DNA sequences encoding HTLV-III core proteins and envelope glycoproteins, respectively. They also include the polypeptides which are produced by translation of the recombinant DNA sequences specific to the Px genes of HTLV-III. The polypeptides may be used as vaccines for the prevention of AIDS. The methods of producing the polypeptides are also a subject of this invention, as are diagnostic methods based on these polypeptides.

Third, the present invention also relates to antibodies against the immunoreactive HTLV-III polypeptides which are the subject of this invention. These antibodies are the basis for assays

relating to the diagnosis of AIDS or the presence of HTLV-III in body fluids.

In one embodiment of this invention, genetic engineering methods are used to isolate DNA sequences of HTLV-III which encode immunoreactive HTLV-III polypeptides, such as the core protein and the envelope glycoprotein, and to identify the nucleotides which comprise those sequences. The proviral genes integrated into host cell DNA are molecularly cloned and the nucleotide sequences of the cloned provirus is determined.

An E. coli expression library of HTLV-III DNA is constructed; in this library are vectors harboring HTLV-III DNA sequences. The HTLV-III genome is cloned and cuts are then made in the cloned HTLV-III genome with restriction enzymes to produce DNA fragments. (Figures 1 and 2) HTLV-III DNA fragments of approximately 200-500bp are isolated from agarose gel, end repaired with T_4 polymerase and ligated to linker DNA. The linker ligated DNA is then treated with a restriction enzyme, purified from agarose gel and cloned in an expression vector. Examples of the expression vectors used are: OmpA, pIN (A,B and C), lambda pL, T7, lac Trp, ORF and lambda gt11. In addition, mammalian cell vectors such as pSV28pt, pSV2neo, pSVdhfr and VPV vectors, and yeast vectors, such as GALI and GAL10, may be used.

The bacterial vectors contain the lac coding sequences, into which HTLV-III DNA can be inserted for the generation of B-galactosidase fusion protein. The hybrid molecules are then introduced into bacteria (e.g., E.coli); those cells which take up a

vector containing HTLV-III DNA are said to be transformed. The bacteria are plated on top of MacConkey agar plates in order to verify the phenotype of clone. If functional B-galactosidase is being produced, the colony will appear red.

Bacterial colonies are also screened with HTLV-III DNA probes containing the DNA regions of interest (e.g., HTLV-III gag and env DNA sequences). This results in identification of those clones containing the insert. Clones which are positive when screened with the DNA probe and positive on the MacConkey agar plates are isolated.

This identification of cells harboring the HTLV-III DNA sequences makes it possible to produce HTLV-III polypeptides which are immunoreactive with HTLV-III specific antibody. The cells from the selected colonies are grown in culture under conditions conducive to allowing the expression of the hybrid protein. The culture is spun down and the resulting cell pellet broken. The total cellular protein is analysed by being run on an SDS polyacrylamide gel. The fusion proteins are identified at a position on the gel which contains no other protein. (Figure 2) Western blot analyses are also carried out on the clones which screened positive. Such analyses are carried out using serum from AIDS patients, with the result that it is possible to identify those clones expressing HTLV-III env-B-galactosidase fusion proteins (antigens) that cross-react with the HTLV-III specific antibody.

In another embodiment of this invention, lambda $_{10}$ clones harboring HTLV-III DNA are cloned

from the replicated form of the virus. As the retrovirus is replicating, double stranded DNA is being produced. Cuts are made in the cloned HTLV-III DNA with the restriction enzyme SstI. (Figure 1a) Because there are two SstI recognition sites within the LTR of HTLV-III DNA, one LTR region is not present in the cloned DNA sequence removed from the $lambda_{10}$ vector. As a result, a small (approximately 200 bp) fragment of the HTLV-III DNA is missing.

The resulting DNA is linearized and fragments are produced by digesting the linearized genomic DNA spanning the env gene region with restriction enzymes. For example, fragments are produced using Kpn or EcoRI plus HindIII, as shown in Figure 1b. The resulting 2.3kb KpnI-KpnI fragments; 1.0kbEcoRI-EcoRI fragments and 2.4Kb EcoRI-HindIII fragments are isolated by gel electrophoresis and electro-elution. These fragments are randomly sheared to produce fragments. The fragments thus produced are purified from agarose gel and DNA fragments between about 200-500 bp are eluted.

The eluted 200-500bp DNA fragments are end filled through the use of E. coli T_4 polymerase and blunt end ligated into an open reading frame expression (ORF) vector, such as pMR100. This ligation may occur at the SmaI site of the pMR100 vector, which contains two promoter regions, hybrid coding sequences of lambdaCI gene and lacI-LacZ gene fusion sequence. In the vector, these are out of frame sequences; as a result, the vector is nonproductive. The HTLV-III DNA is inserted into the vector; the correct DNA fragments will correct the reading

frame, with the result that CI-HTLV-III-B-galactosidase fusion proteins are produced. The expression of the hybrid is under the control of the lac promoter.

Based on the sequence of pMR100, it appears that if a DNA fragment insert cloned into the SmaI site is to generate a proper open reading frame between the lambdaCI gene fragment and the lac-7 fragment, the inserted DNA must not contain any stop codons in the reading frame set by the frame of the lambdaCI gene.

The hybrid molecules are then introduced into E. coli. The bacteria are plated on MacConkey agar plates to verify the phenotype of the clone. If functional B-galactosidase is being produced, the colony will appear red. The colonies are also screened with HTLV-III DNA probes, for the purpose of identifying those clones containing the insert. Clones which are positive when screened with the DNA probe and positive on the MacConkey agar plates are isolated.

The cells from the selected colonies are grown in culture. The culture is spun down and the cell pellet broken. Total cellular protein is analysed by being run on an SDS polyacrylamide gel. The fusion proteins are identified at a position on the gel which contains no other protein. (Figure 4)

Western blot analyses are also carried out on the clones which screened positive. Sera from AIDS patients are used, thus making it possible to identify those clones which express the HTLV-III-env-B-galactosidase fusion proteins (antigens) that cross-react with the HTLV-III specific antibody.

1000 clones were screened by this method; 6 were positive.

Because of the nature of the pMR100 cloning vehicle, a productive DNA insert should also be expressed as a part of a larger fusion polypeptide. HTLV-III env gene containing recombinant clones was identified by colony hybridization. The production of larger fusion polypeptides bearing functional B-galactosidase activity was verified by phenotype identification on MacConkey agar plates; by B-galactosidase enzyamatic assays and by analysis on 75% SDS-polyacrylamide gels. Immunoreactivity of the larger protein with antibody to HTLV-III was assessed by western blot analysis using serum from AIDS patients. These large fusion proteins also reacted with anti-B-galactosidase and anti-CI antiserum. This finding is consistent with the hypothesis that they are proteins of CI-HTLV-III-lacIZ.

The open reading frame insert fragment of HTLV-III is further analyzed by DNA sequencing analysis. Because one of the two BamHI sites flanking the SmaI cloning site in pMR100 is destroyed in the cloning step, positive clones are digested with restriction enzymes HindIII and claI to liberate the inserted HTLV-III DNA fragment. The HTLV-III ORF inserts are isolated from the fusion recombinant and cloned into M13 sequencing cloning vector mp18 and mp19 digested with HindIII and AccI. DNA sequences of the positive ORF clones are then determined.

In another embodiment of this invention, fragments of HTLV-III DNA of approximately 200-500

bps are isolated from agarose gel, end repaired with T_4 polymerase and ligated to EcoRI linker. The EcoRI linker ligated DNA is then treated with EcoRI purified from 1% agarose gel and cloned in an expression vector, gt11. This vector contains lac Z gene coding sequences into which the foreign DNA can be inserted for the generation of B-galactosidase fusion protein. The expression of the hybrid gene is under the control of lac repressor. The lac repressor gene, lac I, is carried on a separate plasmid pMC9 in the host cell, E. coli Y1090. AIDS patient serum was used to probe the gt11 library of HTLV-III genome DNA containing 1.5×10^4 recombinant phage. In a screen of 5000 recombinants, 100 independent clones that produced strong signals were isolated. The positive recombinant DNA clones were further characterized for their specific gene expression. Rabbit hyperimmune serum against P24 was also used to identify the gag gene specific clones. Nick-translated DNA probes of specific HTLV-III gene, specifically the gag gene, env gene and Px gene were used to group the positive immunoreactive clones into specific gene region.

Recombinant clones that produced strong signals with AIDS serum and contain insert DNA spanning the HTLV-III env gene region were examined in detail by mapping their insert with restriction enzymes and DNA sequencing analysis.

Another embodiment of this invention relates to the formation of RNA and RNA probes specific to the HTLV-III DNA of this invention. DNA sequences which are an entire gene or segment of a gene from HTLV-III are inserted into a vector, such as a T7 vector.

In this embodiment, the vector has the Tceu promoter from the T cell gene 10 promoter and eleven amino acids from the T cell gene 10 protein.

The vectors are then used to transform cells, such as E. coli. The T7 vector makes use of the T7 polymerase, which catalyzes RNA formation and recognizes only T7 promoter, which is the site where RNA polymerase binds for the initiation of transcription. This vector does not, however, recognize E. coli promoter. As a result, if HTLV-III DNA sequences are inserted after the promoter and polymerase genes of the T7 vector, which recognizes them to the exclusion of other signals, and a terminator is placed immediately after the HTLV-III DNA sequences, the T7 vector will direct manufacture RNA complementary to the HTLV-III DNA insert.

Monoclonal antibodies reactive with HTLV-III envelope polypeptide are produced by antibody-producing cell lines. The antibody-producing cell lines may be hybridoma cell lines commonly known as hybridomas. The hybrid cellsa re formed from the fusion of cells which produce antibody to HTLV-III envelope polypeptide and an immortalizing cell line, that is, a cell line which imparts long term tissue culture stability on the hybrid cell. In the formation of the hybrid cell lines, the first fusion partner - the antibody-producing cell - may be a spleen cell of an animal immunized against HTLV-III envelope polypeptide. Alternatively, the antibody-producing cell may be an anti-HTLV-III envelope polypeptide lymphocyte obtained from the spleen, peripheral blood, lymph nodes or other tissue. The

second fusion partner - the immortal cell - may be a lymphoblastoid cell or a plasmacytoma cell such as a myeloma cell, itself an antibody-producing cell but also malignant.

Murine hybridomas which produce monoclonal antibodies against HTLV-III envelope polypeptide are formed by the fusion of mouse myeloma cells and spleen cells from mice immunized against the polypeptide. To immunize the mice, a variety of different immunization protocols may be followed. For instance mice may receive primary and boosting immunizations of the purified polypeptide. The fusions are accomplished by standard procedures. Kohler and Milstein, (1975) Nature (London) 256, 495-497; Kennet, R., (1980) in Monoclonal Antibodies (Kennet et al., Eds. pp. 365-367, Plenum Press, NY).

The hybridomas are then screened for production of antibody reactive with envelope polypeptide.

Another way of forming the antibody-producing cell line is by transformation of antibody-producing cells. For example, a B lymphocyte obtained from an animal immunized against HTLV-III envelope polypeptide may be infected and transformeed with a virus such as the Epstein-Barr virus in the case of human B lymphocytes to give an immortal antibody-producing cell. See, e.g., Kozbor and Rodor (1983) Immunology Today 4(3), 72-79. Alternatively, the B lymphocyte may be transformed by a transforming gene or transforming gene product.

The monoclonal antibodies against HTLV-III envelope polypeptide are produced in large quantities by injecting antibody-producing hybridomas into the peritoneal cavity of mice and, after an

appropriate time, harvesting the ascites fluid which contains very high titer of homogenous antibody and isolating the monoclonal antibodies therefrom. Xenogeneic hybridomas should be injected into irradiated or athymic nude mice. Alternatively, the antibodies may be produced by culturing cells which produce HTLV-III envelope polypeptide in vitro and isolating secreted monoclonal antibodies from the cell culture medium.

This invention will now be further illustrated by the following examples. They are not intended to be limiting in any way.

EXAMPLE 1
PREPARATION OF SONICATED DNA FRAGMENTS

10 ug of gel purified HTLV-III restriction fragments were sonicated to fragment size on average of 500 bps. After sonication, the DNA was passed through a DEAE-cellulose column in 0.1XTBE in order to reduce the volume. The DEAE-bound DNA was washed with 5 ml of 0.2 M NaCl-TE (2 M NaCl, 10 mm Tris HCl pH 7.5, 1 mM EDTA) and then eluted with 1 M NaCl-TE, and ethanol precipitated. The size range of the sonicated DNA was then determined on 1.2% agarose gel. DNA fragments of desired length (200-500 bps) was eluted from the gel. T4 DNA polymerase was used to fill in and/or trim the single strand DNA termini generated by the sonication procedure. DNA fragments were incubated with T4 polymerase in the absence of added nucleotides for five minutes at 37°C to remove nucleotides from 3' end and then all 4 nucleotide precursors were added to a final

concentration of 100 uM and the reaction mixture was incubated another 30 minutes to repair the 5'-end single stranded overhang. The reaction was stopped by heat inactivation of the enzyme at 68°C for 10 minutes. DNA was phenol extracted once, ethanol precipitated and resuspended in TE.

EXAMPLE 2

CLONING OF RANDOM SHEARED DNA FRAGMENTS

The sonicated blunt end repaired HTLV-III DNA fragments were ligated into the SmaI site of the ORF expression vector pMR100 and transformed into host cell LG90 using standard transformation procedures. B-galactosidase positive phenotype of the transformant were identified by plating the transformed cell on ampicillin (25 ug/ml) containing McConkey agar plates and scoring the phenotype after 20 hours at 37°C.

EXAMPLE 3

HYBRID PROTEIN ANALYSIS

Ten milliliter samples of cells from an overnight saturated culture grown in L broth containing ampicillin (25 ug/ml) were centrifuged, the cell pellet was resuspended in 500 ul of 1.2 fold concentrated Laemmli sample buffer. The cells were resuspended by vortexing and boiling for 3 minutes at 100°C. The lysate was then repeated by being forced through a 22 guage needle to reduce the lysate viscosity. Approximately 10 ul of the protein samples were electrophoresed in 7.5% SDS-PAGE (SDS-polyacrylamide) gels.

Electrophoretic transfer of proteins from SDS-PAGE gels to nitrocellulose paper was carried out according to Towbin et. al.. After the transfer, the filter was incubated at 37°C for two hours in a solution of 5% (w/v) nonfat milk in PBS containing 0.1% antifoam A and 0.0001% merthiolate to saturate all available protein binding sites. Reactions with AIDS antisera were carried out in the same milk buffer containing 1% AIDS patient antisera that had been preabsorbed with E. coli lysate. Reactions were performed in a sealed plastic bag at 4°C for 18-24 hours on a rotatory shaker. Following this incubation, the filter was washed three times for 20 minutes each at room temperature in a solution containing 0.5% deoxycholic, 0.1 M NaCl, 0.5% triton X-100, 10 mm phosphate buffer pH 7.5 and 0.1 mM PMSF.

To visualize antigen-antibody interactions, the nitrocellulose was then incubated with the second goat antihuman antibody that had been iodinated with ^{125}I. The reaction with the iodinated antibody was carried out at room temperature for 30 minutes in the same milk buffer as was used for the first antibody. The nitrocellulose was then washed as previously described and exposed at -70°C using Kodak XAR5 film with an intensifying screen.

EXAMPLE 4

SCREENING OF THE HTLV-III ORF LIBRARY BY COLONY HYBRIDIZATION

E. coli LG90 transformants were screened with HTLV-III DNA probes containing the DNA regions of interest (e.g. HTLV-III gag, env or Px gene specific

sequences). Colonies were grown on nitrocellulose filter and screened according to the procedure of Grunstein and Hogness by using a nick-translated HTLV-III DNA as hybridization probe.

The DNA fragment was in general exercise by restriction endonuclease digestion, gel purified, and ^{32}P-labeled to a specific activity of 0.5×10^8 cpm/ug by nick-translation (Rigby, P.W.J. et al., J. Mol. Biol. 113, 237 (1977). Duplicate nitrocellulose filters with DNA fixed to them were prehybridized with 6xSSC (0.9 M NaCl/0.09 M sodium citrate, pH 7.0), 5X Denhardt's solution (Denhardt's solution: 0.02% each of polyvinylpyrrolidone, Ficoll and bovine serum albumin) 10 ug of denatured sonicated E. coli DNA per ml at 55°C for 3-5 hours. The filters were then placed in a fresh sample of the same solution to which the denatured hybridization probe had been added. Hybridization was permitted to take place at 68°C for 16 hours. The filters were washed repeatedly in 0.3XSSC at 55°C, and then exposed to x-ray film.

Industrial Applicability

This invention has industrial applicability in screening for the presence of HTLV-III DNA in body fluids and the diagnosis of AIDS.

Equivalents

Those skilled in the art will recognize, or be able to ascertain, using no more than routine experimentation, numerous equivalents to the specific substantces and procedures described herein. Such equivalents are considered to be within the

scope of this invention and are covered by the following claims.

CLAIMS

1. Immunoreactive HTLV-III polypeptide expressed by cells transformed with a recombinant vector containing HTLV-III cDNA.

2. A polypeptide of Claim 1 wherein said HTLV-III cDNA encodes an *env* gene sequence.

3. A polypeptide of Claim 2 wherein which is immunoreactive with sera of patients with acquired immunodeficiency syndrome.

4. Isolated HTLV-III envelope polypeptide.

5. Isolated cDNA encoding an HTLV-III gene.

6. cDNA of Claim 5 encoding the HTLV-III *env* gene.

7. Isolated cDNA encoding for an HTLV-III polypeptide which is immunoreactive.

8. Isolated cDNA of Claim 7 coding for an envelope polypeptide which is immunoreactive.

9. A DNA probe comprising a DNA sequence coding a portion of the HTLV-III genome.

10. A DNA probe of Claim 9 wherein the DNA sequence encodes at least a portion of the *env* gene.

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11. A hybrid protein comprising an HTLV-III polypeptide linked to at least one other polypeptide.

12. A hybrid protein of Claim 11 comprising an HTLV-III polypeptide linked to an indicator polypeptide.

13. A hybrid protein of Claim 12 wherein said indicator polypeptide comprises beta-galactosidase.

14. An isolated RNA transcript of the env gene of HTLV-III.

15. An isolated RNA transcript of Claim 14 having a label which emits a detectable signal.

16. An isolated RNA transcript of Claim 15 wherein said label comprises a radioisotope.

17. A recombinant vector containing HTLV-III DNA capable of expression upon insertion into host cells.

18. OmpA vector containing HTLV-III cDNA.

19. pMR 100 vector containing HTLV-III cDNA.

20. A method of producing HTLV-III polypeptide, comprising the steps of:

    a. cleaving HTLV-III cDNA to produce DNA fragments;

b. inserting the DNA fragments into an expression vector to form a recombinant vector;

c. transforming an appropriate host cell with the recombinant vector; and

d. culturing the transformed host cell under conditions sufficient for expression of the polypeptide coded for by the inserted HTLV-III DNA.

21. A method of Claim 20 wherein the cleaving step comprises digesting the HTLV-III cDNA with restriction endonucleases to produce restriction fragments of cDNA.

22. A method of Claim 20 wherein the cleaving step comprises shearing the HTLV-III cDNA to produce cDNA fragments.

23. A method of producing HTLV-III envelope polypeptide, comprising the steps of:

a. cleaving HTLV-III genomic cDNA with the restriction endonuclease SstI;

b. digesting the cleaved cDNA with restriction endonucleases sufficient to generate restriction fragments which encompass at least a portion of the env gene;

c. isolating the restriction fragments;

d. producing DNA fragments of about 200-500 base pairs in length from the restriction fragments;

e. isolating the DNA fragments of about 200-500 base pairs;

f. inserting the isolated fragments into the open reading frame expression vector pMR100 for production of hybrid proteins comprising an env gene product and beta-galactosidase;

g. transforming lac z⁻ E. coli cells with the vector;

h. plating the transformed cells on MacConkey agar plates, maintaining the plates under conditions sufficient for the formation of colonies and selecting cell colonies exhibiting a red color;

i. culturing transformed cells from the selected colonies under conditions which allow expression of the hybrid protein;

j. obtaining cellular protein from the cultured transformed cells;

k. separating the cellular protein obtained;

l. contacting the separated protein with sera from AIDS patients to identify protein which is immunoreactive with the sera; and

m. isolating the immunoreactive protein.

/

24. A method of Claim 23, further comprising the step of separating the env gene expression product from the remainder of the hybrid protein.

25. A fusion protein produced by the method of Claim 23./

26. A HTLV-III envelope polypeptide produced by the method of Claim 24.

27. Antibody specifically reactive with HTLV-III envelope polypeptide.

28. An antibody of Claim 27 which is monoclonal.

29. Anibody specifically reactive with HTLV-III polypeptide produced by recombinant DNA techniques.

30. An antibody of Claim 29 which is monoclonal.

31. An immunoassay for the detection of HTLV-III employing antibody which reacts specifically with HTLV-III polypeptide produced by recombinant DNA techniques.

32. An immunoassay for the detection of HTLV-III employing antibody which reacts specifically with HTLV-III envelope polypeptide.

33. An immunoassay of Claim 32 wherein said antibody is monoclonal.

34. A method for detecting the presence of HTLV-III in a bodily fluid comprising the steps of:

a. contacting an immunoadsorbent comprising a solid phase having an antibody which specifically binds HTLV-III polypeptide with the bodily fluid;

b. separating the immunoadsorbent and the fluid;

c. contacting the immunoadsorbent with a labeled antibody which specifically binds HTLV-III polypeptide; and

d. measuring the amount of label associated with the immunoadsorbent to determine the presence of HTLV-III.

35. An assay kit comprising an antibody which reacts specifically iwth HTLV-III polypeptide bound to a solid phase and a labeled antibody which reacts specifically HTLV-III polypeptide.

36. A method of determining the presence of antibodies against HTLV-III in a bodily fluid comprising the steps of:

a. contacting an immunoadsorbent comprising an HTLV-III polypeptide bound to a solid phase with a bodily fluid;

b. separating the immunoadsorbent from the bodily fluid;

c. contacting the immunoadsorbent with a labeled HTLV-III polypeptide; and

d. determining the amount of labeled polypeptide bound to immunoadsorbent as an indication of antibody to HTLV-III.

37. A kit for determining the presence of antibody against HTLV-III in a bodily fluid comprising:

a. an immunoadsorbent comprising a HTLV-III polypeptide bound to a solid phase; and

b. labeled HLTV-III polypeptide.

38. A method of detecting HTLV-III nucleic acid in a bodily fluid comprising the steps of:

a. adsorbing the nucleic acid in a bodily fluid onto an adsorbent;

b. denaturing the adsorbed nucleic acid;

c. contacting the adsorbed nucleic acid with a HTLV-III DNA or RNA probe; and

d. determining if the probe hybridizes with the adsorbed nucleic acid.

39. A method of Claim 38 wherein the bodily fluid is a cell lysate.

40. A hybridoma cell line which produces antibody specifically reactive with HTLV-III envelope polypeptide.

CLONING AND EXPRESSION OF HTLV-III DNA

Abstract

The production of immunoreactive polypeptides from HTLV-III by recombinant DNA methods is disclosed. Such polypeptides can be employed in immunoassays to detect HTLV-III.

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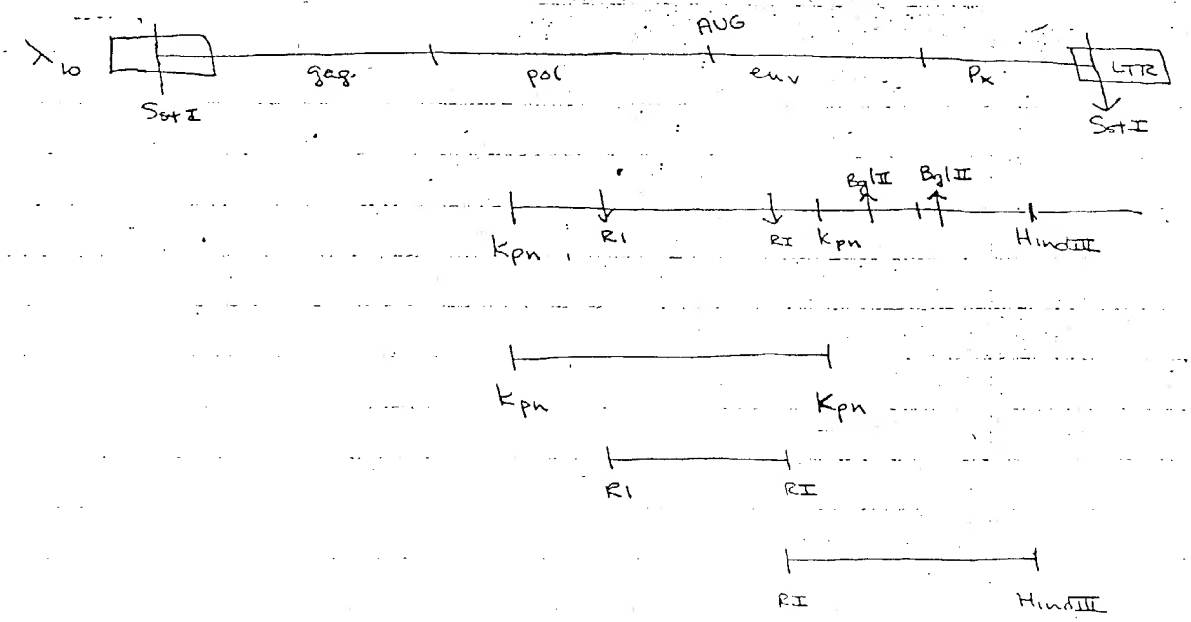
FIGURE 1



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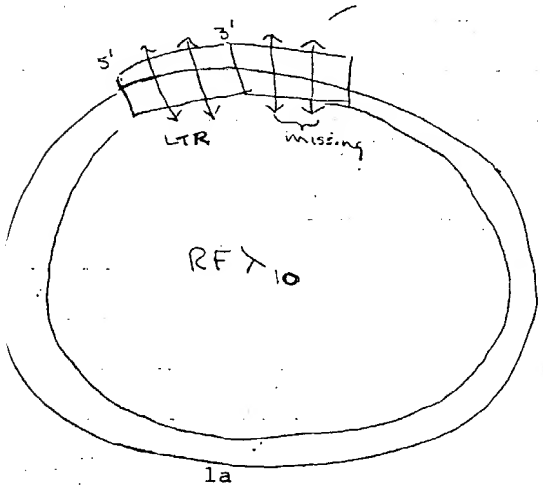

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FIGURE 2

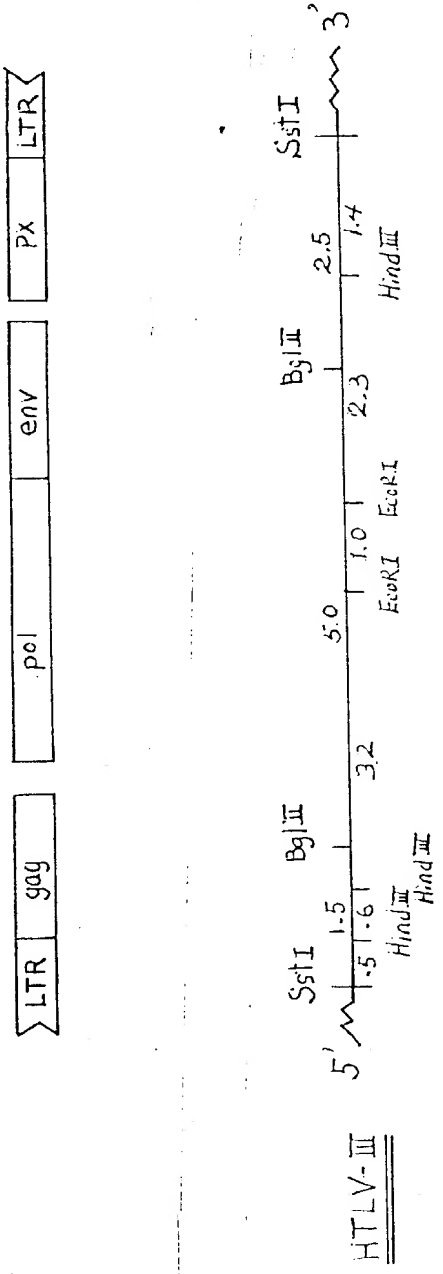
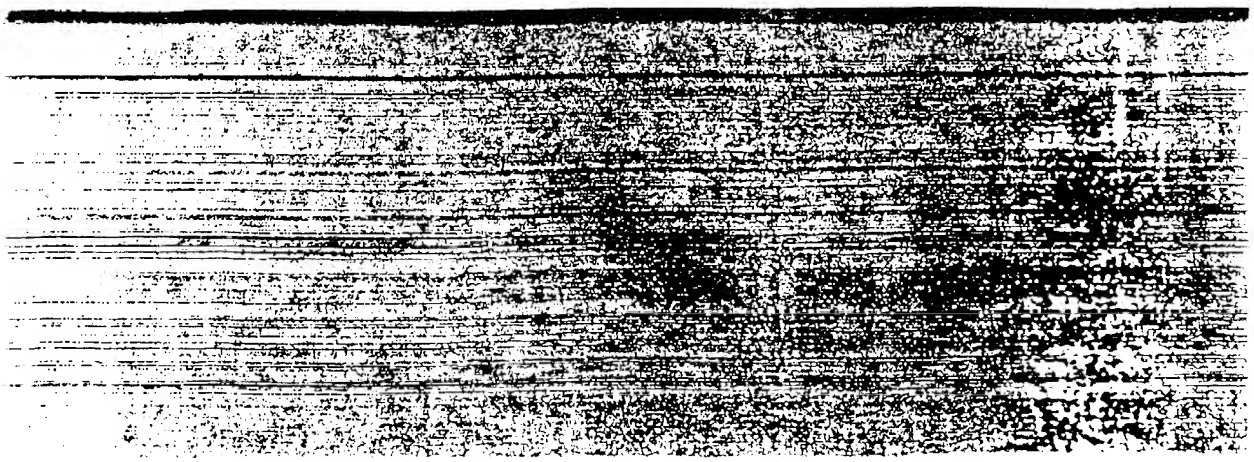


FIGURE 3 (A)



(B)

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 990       1000      1010      1020      1030      1040      1050
TCTGGAAAGC TGAAGGGGCA CTAGCTAATAC AAGATCGATAG TGACAT[illegible]AA GTAGTGCCAA CAAGAAGAGC

1060      1070      1080      1090      1100      1110      1120
AGAGATCATT ACCGATTATG GAAAACAGAT GGCAGGTGAT GATTGTGTGG CAAGTAGGGA CGATGAGGAT

1130      1140      1150      1160      1170      1180      1190
TAGAACATGG AAAACTTTAG TAAAACAGCA TATGTATGTT TCAGGGAAAG CTAGGGGATG GTTTTATAGA

1200      1210      1220      1230      1240      1250      1260
GATCACTATC AAAGCCCTCA TCCAAGAATA AGTTCAGAAG TACACATCCC ACTAGGGGAT GCTAGATTGG

1270      1280      1290      1300      1310      1320      1330
TAATAAGAGC ATATTTGGGT CTGCATACAG GAGAAAGAGA CTGGCATTTG GGTCAGGCAG TCTCCATAGA

1340      1350      1360      1370      1380      1390      1400
ATGGACGAAA AAGACATATA GCACACAAGT AGACCCTGAA CTAGCAGCCC AACTAGTTCA TCTGTATTAC

1410      1420      1430      1440      1450      1460      1470
TTTGACTGTT TTTCAGACTC TGCTATAAGA AAGGCTTAT TAGGACACAT AGTTAGCCCT AGGTGTGAAT

1480      1490      1500      1510      1520      1530      1540
ATCAAGCAGG ACATAACAAG GTAGGATCTC TACAATACTT GGCACTAGCA GCATTAATAA CACGAAAAGA

1550      1560      1570      1580      1590      1600      1610
GATAAAGCCA CTTTTGCCTA GTGTTACTCA ACTGACAGAG GATACATGGA ACAAGCCCCA GAAAGACCAA

1620      1630      1640      1650      1660      1670      1680
GGCCCACAGA GCGAGCCACA CAATGAATGG ACACTAGAGC TTTTAGAGGA GCTTAAGAAT GAAGCTGTTA

1690      1700      1710      1720      1730      1740      1750
CACATTTTCC TAGGATTTGG CTCCATGGCT TAGGGCAACA TATCTATGAA ACTTATGGGG ATACTTGGGC

1760      1770      1780      1790      1800      1810      1820
AGGAGTGGAA GCCATAATAG GAATTCTGCA ACAACTGCTG TTTATCCATT TTCAGAATTG GGTGTCGACA

1830      1840      1850      1860      1870      1880      1890
TAGCAGAATA GGCGTTACTC GACAGAGGAG AGCAAGAAAT GGAGCCAGCA GATCCTAGAC TAGAGCCCGC

1900      1910      1920      1930      1940      1950      1960
AAGCATCCAG GAAGTCAGCC TAAAACTGCT TGTACCAATT GCTATTGTAA AAAGTGTTGC TTTCATTGCC

1970      1980      1790      2000      2010      2020      2030
AAGTTTGTTT CATAACAAAA GCCTTAGGCA TCTCCTATGG CAGGAAGAAG CGGAGACAGC GACGAAGAGC

2040      2050      2060      2070      2080      2090      2100
GCCTCAAGGC AGTCAGACTC ATCAAGTTTC TCTATCAAAG CAGTAAGTAG TACATGTAAT GCAACCTATA

2110      2120      2130      2140      2150      2160      2170
CAAATAGCAA TACTAGCATT AGTAGTAGCA ATAATAATAG CAATAGTTGT GTGGTCCATA GTAATCATAG

2180      2190      2200      2210      2220      2230      2240
AATATAGGAA AATATTAAGA CAAAGAAAAA TAGACAGGTT AATTGATAGA CTAATAGAAA [illegible]

2250      2260      2270      2280      2290      2300      2310
CAGTGGCAAT GAGAGTGAAG GAGAAATATC AGCACTTGTG CAGATGGGGC TGGAGAT[illegible] [illegible]

2320      2330      2340      2350      2360      2370      2380
[illegible]TGGATTATCT GTACTGCTAC AGAAAAATTG TGGGTCACAG TCTATT[illegible] [illegible]
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2390 2400 2410 2420 2430 2440 2450
TGGAAGCAAG CAACCACCAC TCTATTTTGT GCATCAGATG CTAAAGCATA TGATACAAGA GGTACATAAT
2460 2470 2480 2490 2500 2510 2520
GTTTGGGCCA CACATGCCTG TGTACCCACA GACCCCAACC CACAAGAAGT AGTATTCGTA AGTGTGACAG
2530 2540 2550 2560 2570 2580 2590
AAAATTTTAA CATGTGCAAA AATGACATGG TAGAACAGAT GCATGAGGAT ATAATCATTT TATGGGATCA
2600 2610 2620 2630 2640 2650 2660
AACCCTAAAG CCATGTGTAA AATTAACCCC ACTCTGTGTT AGTTTAAAGT GCACTCTTTT GAAGAGTGAT
2670 2680 2690 2700 2710 2720 2730
ACTAATACCA ATAGTACTAG CGGGAGCATG ATAATGCAGA AAGCAGCAGT GAAAAACTGC TCTTTCAGTA
2740 2750 2760 2770 2780 2790 2800
TCAGCACAAG CATAAGAGGT AAGGTCCAGA AAGAATATCC ATTTTTTTAT AAACTTGATA TAATACCAGT
2810 2820 2830 2840 2850 2860 2870
AGATAGTGAT ACTACCAGCT ATACGTTGAC AAGTTGTAAC ACCTCAGTCA TTACACATGC CTGTCCAAAG
2880 2890 2900 2910 2920 2930 2940
GTATCCTTTG AGCCAATTCC CATACATTAT TGTGCCCCCG CTGGTTTTGC GATTCTAAAA TGTAATAATA
2950 2960 2970 2980 2990 3000 3010
AGACGTTCAA TGGGACAGCA CCATGTACAA ATCTCAGCAC AGTTCCGATG TCACACATGG AA TTAGCCAGT
3020 3030 3040 3050 3060 3070 3080
AGTATCAACT CAACTGCTGT TAAATGCCAG TCTACCAGAA GAAGAGGTAG TAATTAGATC TGTCAATTTC
3090 3100 3110
ACGGACAATG CTAAAACCAT AATAGTACAG CT

FIGURE 4

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